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Antimicrobial varnish and root surface caries

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SUMMARY

The original intent of this thesis was to determine the potential inhibitory effect of an antimicrobial-releasing varnish on root surface demineralisation *in situ*. This varnish (Cervitec[®]) was designed in order to deal with a major caries problem in clinical dentistry today and in the future: root surface caries. Previously published studies on antimicrobial-releasing varnishes have been focussed in particular on the microbiological aspects of plaque- and specific bacteria reductions. No quantitative microradiographical data have been reported on the efficacy of antimicrobial varnishes on dental hard tissue demineralisation. Initially, the efficacy of the varnish on dentin was investigated. After promising results, the efficacy on enamel was incorporated in this work.

Primary aims of this thesis, as described in chapter 1, were:

- To study the efficacy of an antimicrobial-containing varnish *in situ* on root surface demineralisation.
- To investigate the release of the two antimicrobials, chlorhexidine (1%^{w/w}) and thymol (1%^{w/w}) from this varnish system.

Secondary aims were:

- To study the efficacy of the varnish *in situ* on enamel demineralisation.
- To investigate and describe the adsorption of chlorhexidine to dental hard tissues.

Chapter 2 gives a survey of the literature pertaining to root surface caries, antimicrobial agents, varnishes and a brief discussion of enamel caries.

Root surface caries is predominantly confined to teeth with exposed roots, a situation which is in most cases the result of periodontal breakdown. After inadequate oral hygiene and frequent sugar intake, pathogenic plaque action will cause periodontal inflammation, which induces gingival recession, alveolar bone loss and ultimately leads to accessible roots. Root caries primarily affects mature people and its frequency is increasing due to increased life expectancy and dentition retention. The histopathology of root caries is still partly unknown because of the complex de- and remineralisation processes which occur in dentin. Bacteria implicated among others in root caries are mutans streptococci, *Actinomyces* spp. and also *Lactobacillus* spp.

Chlorhexidine, though one of the strongest antimicrobials used in the oral cavity, is widely employed because of its broad spectrum activity against gram-negative but especially against gram-positive bacteria. Mutans streptococci seems to be particularly sensitive. Negative side-effects of regular use of high concentrations of chlorhexidine are dark staining of teeth and mucosa, alterations in taste sensation and lesions (desquamations) on the oral mucosa.

Thymol is the other well accepted antimicrobial incorporated in the varnish used in this work and is reported to have a minor *in vivo* antimicrobial efficacy.

Dental varnishes have been previously used as fluoride carriers. Varnishes have the advantages of low dosages of active ingredients, a localized activity (at the site where it is needed) and a prolonged contact time. The antiplaque effects of antimicrobial varnishes have been recently investigated. These studies demon-

strate that the chlorhexidine-containing varnishes had a strong inhibiting effect especially on mutans streptococci and showed little negative side-effects.

In *chapter 3* root surface demineralisation *in situ* is presented after varnish applications. The varnish, with or without active ingredients chlorhexidine and thymol, was applied once or twice on the root surface prior to a two-week *in situ* demineralisation period. Ten participants carried sound, intact root samples in lower dentures for 4 consecutive 2-week periods in a randomized cross-over design. The application of varnish containing active agents resulted in a statistically significant demineralisation reduction. Reductions of about 80% for lesion depth and mineral loss were measured. The varnish base (without active agents) had no effect.

The efficacy of the varnish on root surface demineralisation as a function of the demineralisation period is presented and discussed in *chapter 4*. The results show that the caries reduction in the presence of the varnish was significant up until 2 weeks, but decreased in magnitude with the length of the *in situ* demineralisation period. After 4 and 6 weeks there were no longer statistically significant differences with respect to no treatment. This could be due to the severe demineralisation conditions in this intra-oral model.

The results of the experiments described in chapters 3 and 4 raised the question whether the varnish would also be effective in enamel caries reduction. In *chapter 5* the same experimental set-up as presented above was used with enamel. The results show a statistically significant reduction in the lesion depth (reduction of about 50%) for upto 4 weeks while the mineral loss was reduced up to 2 weeks (reduction of about 60%). Differences between lesion progress in dentin and in enamel are discussed. Demineralisation of dentin occurs at twice the rate seen in enamel. Lesion depth and mineral loss in enamel were *in situ* proportional to the demineralisation period in contrast to prior *in vitro* investigations.

In *chapter 6* the *in vitro* release of chlorhexidine and thymol from the varnish system is described. U.V. spectrophotometry was employed as measuring technique, using a 2 component analysis for the varnish system containing both antimicrobials. The results show that:

- 1) agent release takes place for at least 3 months.
- 2) the amount of chlorhexidine diffusing out of the component varnish is dependent on the presence of thymol.

In *chapter 7* the adsorption of chlorhexidine on powdered dentin, enamel and hydroxyapatite (HAP) as well as on intact dentin and enamel is presented. The maximum binding capacity (B_{max}), affinity constant (K) and the fraction of covered adsorption sites is estimated and discussed assuming a Langmuir adsorption model. For powdered substrates the chlorhexidine uptake can be ranked as: dentin >> enamel > HAP and in intact substrates dentin >> enamel.

The affinity constant K is $\pm 35 \text{ L.g}^{-1}$ in powdered and intact dentin. Furthermore, K (dentin) is smaller than K (enamel) and K (HAP). For powdered dentin

and enamel respectively.
It is concluded:
1) chlorhex
2) chlorhex

In *chapter 8* the results of the experiments described in *chapter 8* are discussed.
1) Differences between *in situ* and *in vitro* conditions described in *chapter 8* are discussed in terms of limited information, costs, accuracy, demineralisation. Presently, the results are under consideration. Recently, the results are reported and discussed.
2) Efficacy of the varnish. Results of a study are presented. It is concluded that the varnish is effective in reducing caries, but not as effective as already some other treatments. The varnish is effective on roots.
3) The mechanism of action. The varnish is firstly into the dentin, then into the enamel. The varnish adheres to the dentin. The point mentioned in the text is estimated. The antimicrobial effect is unknown. It is concluded that the varnish is effective in reducing caries in roots.
4) Clinical use. The varnish is used for clinical use.
5) Future varnishes. The varnish is considered.
From this the results are concluded. The varnish is likely to reduce caries, but not as likely as other treatments.

and enamel B_{max} values were ± 1.3 mg and 0.3 mg per gram material, respectively, equivalent to about 0.4 and 0.03 mg. m^{-2} .

It is concluded that:

- 1) chlorhexidine binds mainly to the organic matrix in dentin and in enamel.
- 2) chlorhexidine can diffuse into the hard tissues.

In *chapter 8*, the general discussion some important aspects of varnish application are discussed:

1) Differences between *in situ* and *in vivo* experiments. The possible differences between *in vivo* and *in situ* studies utilizing samples inserted in dentures as described in this work. Advantages of *in vivo* studies are the realistic "field" conditions obtained in a large group of the population. Disadvantage is the limited information provided. Advantages of *in situ* experimentation are the low costs, accurate measuring techniques, the possibility of creating known demineralisation or remineralisation conditions and the use of adequate statistics. Presently, there are no reasons to assume that the efficacy of the plaque situation under consideration is very different in *in situ* and in *in vivo* conditions.

Recently, the varnish (Cervitec[®]) effect under normal *in vivo* conditions was reported and showed to be 3-4 months.

2) Efficacy of the varnish on sound flattened dentin and on demineralised roots. Results of a separate *in situ* study show that the varnish investigated has potential to reduce caries also in already partially demineralised roots. This is important because it is difficult to estimate clinically whether a root is still sound or already somewhat demineralised.

The varnish was found to be less effective on flattened dentin than on intact roots.

3) The mechanism of varnish action. The varnish releases the antimicrobials firstly into the saliva, into the pellicle and into the hard tissues. Because the varnish adheres to the tissues for considerable periods (at least 2 days), the last point mentioned is important. The penetration depth of chlorhexidine into dentin is estimated in this work to be $\pm 100-300$ μm . The relative importance of the antimicrobials released from the tissue, pellicle and saliva in due time is presently unknown. It is assumed, however, that the presence of chlorhexidine in the dentin and its subsequent release from this tissue are important mechanistic aspects in root caries prevention.

4) Clinical use of antimicrobial-releasing varnishes. Some recommended steps for clinical use of the varnish are listed.

5) Future varnish developments. Some varnish improving possibilities are considered.

From this thesis it can be concluded that antimicrobial releasing varnishes effectively reduce root surface caries as well as enamel caries *in situ*. This material will most likely play an important role in *professional* caries prevention in the future.